PATENT ABSTRACTS OF JAPAN

(11)Publication number : 10-248562

(43)Date of publication of application : 22.09.1998

(51)Int.Cl.

C12N 9/20
C12G 3/02
C12N 9/16
C12N 9/30
C12N 9/34
C12N 9/50
//(C12N 9/20
C12R 1:785)
(C12N 9/20
C12R 1:845)
(C12N 9/20
C12R 1:69)
(C12N 9/20
C12R 1:72)
(C12N 9/20
C12R 1:38)
(C12N 9/20
C12R 1:66)
(C12N 9/20
C12R 1:80)
(C12N 9/16
C12R 1:80)
(C12N 9/16
C12R 1:69)
(C12N 9/30
C12R 1:69)
(C12N 9/34
C12R 1:685)
(C12N 9/50
C12R 1:845)

(21)Application number : 09-074374

(22)Date of filing : 10.03.1997

(71)Applicant : TAX ADM AGENCY
AMANO PHARMACEUT CO LTD

(72)Inventor : IWANO KIMIO
MIKAMI SHIGEAKI
YOKOI NOBUMASA
AMANO HITOSHI

(54) EXOGENOUS ENZYME FOR BREWING AND BREWING BY USING THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain an exogenous enzyme for brewing capable of being used instead of malted rice (Koji) in the brewing of Japanese sake (rice wine), Japanese

shochu (a distilled spirit), Mirin (glutinous rice wine), etc.
SOLUTION: This exogenous enzyme is obtained by formulating lipase and/or nuclease with an exogenous enzyme comprising α-amylase, glucoamylase, acid protease and optionally acid phosphotase, capable of providing a raw material utilization factor equal to or more than the case using a malted rice when used instead of the malted rice, and further capable of providing a brewage qualitatively excellent in sensory flavor. The exogenous enzyme is used instead of the malted rice, manifests activities equal to or more than the malted rice, and effective to enlarge a volume of the production and reduction in labor for the brewing. The exogenous enzyme containing lipase and/or nuclease as an active ingredient is also utilized as a strengthening material of the malted rice.

LEGAL STATUS

[Date of request for examination]	14.11.2000
[Date of sending the examiner's decision of rejection]	11.11.2003
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
[Patent number]	3598361
[Date of registration]	24.09.2004
[Number of appeal against examiner's decision of rejection]	2003-23788
[Date of requesting appeal against examiner's decision of rejection]	09.12.2003
[Date of extinction of right]	

* NOTICES *

JPO and NCIPI are not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technique in which invention belongs] This invention relates to the method of distilling an alcoholic beverage for having used the enzyme agent for a brewing, and it. Furthermore, it is related with the method of distilling the alcoholic beverage using the enzyme agent for a brewing and it which are used for a detail as reinforcement and/or substitution of koji in the case of a brewing of high-class sake, such as quality sake brewed from the finest rice and junmai-shu.
[0002]
[Description of the Prior Art] High-class sake, such as quality sake brewed from the finest rice and junmai-shu, carries out altitude cleaning of the raw material white rice at 60% or less of rice-cleaning rates, builds careful distillation koji by the peculiar koji-making method, will perform low-temperature long-term fermentation for 40 days for raw sake from the 30th at 5 degrees C - 11 degrees C low temperature, and is manufacturing the sake of high quality. However, even if it only cleans raw material rice to altitude and performs low-temperature fermentation, if careful distillation koji is not good, the quality sake brewed from the finest rice which was excellent in quality cannot be built.
[0003] Although the quality of careful distillation koji has had the biggest effect on the quality of high-class alcohol, this koji-making technique is an advanced tradition technique, and is a technique which the skillful chief sake brewer has inherited secretly respectively.
[0004] Moreover, since [which careful distillation koji calls a lid and the **** method] koji making is carried out by the koji-making method of hand structure from ancient times, the quantitative expansion is difficult.
[0005]
[Problem(s) to be Solved by the Invention] Although sale of high-class sake, such as quality sake brewed from the finest rice and junmai-shu, is increasing recently, since the technique which builds the careful distillation koji which was excellent in quality as mentioned above being a skillful advanced technique, and koji making are hand structure and quantitative expansion is difficult for them, they have the trouble that production cannot be increased corresponding to the increment in a distribution cost.
[0006] Moreover, it is necessary to clean raw material white rice to altitude, and since the dissolution of a steamed rice is bad and there is still more ** because of low-temperature fermentation, the trouble that a manufacturing cost is high is accepted.
[0007] As an enzyme agent for raw sake, the enzyme agent which blended alpha-amylase, glucoamylase, acid protease, and acid phosphatase showed clearly that it usually has a function equivalent to koji in a brewing (patent No. 2045841). However, in low-temperature fermentation, it becomes clear that a function equivalent to koji in this enzyme agent cannot be demonstrated, have a function equivalent to careful distillation koji, and it is used instead of reinforcement of koji, or koji. Manufacturing high-class sake, such as quality sake brewed from the finest rice of high quality which was excellent in the flavor, and junmai-shu, and development of the enzyme agent for a brewing which the dissolution of a steamed rice can be further raised in low-temperature fermentation, and can lower a

manufacturing cost were performed.
[0008]
[Means for Solving the Problem] When research was wholeheartedly repeated about the enzyme in connection with the concurrency double fermentation in low-temperature fermentation, by using it in many enzymes, replacing the enzyme agent containing using lipase and/or nuclease for reinforcement of koji or lipase, and/or nuclease with koji, the rate of the dissolution of the steamed rice in low-temperature fermentation could be raised, it found out that the aroma quantitative formula of production alcohol increased further, and this invention was completed.
[0009] That is, this invention is (1). It is the enzyme agent for a brewing which contains the lipase and/or nuclease which are used as reinforcement of koji as an active principle, and is [0010]. (2) It is the enzyme agent for a brewing which contains the lipase and/or nuclease which are used as substitution of koji, and alpha-amylase, glucoamylase and acid protease as an active principle, and also contains acid phosphatase more preferably, and is [0011]. (3) It is a method of distilling the alcoholic beverage characterized by using the enzyme agent for a brewing given in (1) for main fermentation raw sake as reinforcement of koji, and using the enzyme agent for a brewing given in (2) for it as substitution of koji.
[0012] Now, using the lipase which is the active principle of the enzyme agent for a brewing of this invention in a brewing is reported. That is, "the manufacturing method (JP,59-21594,B) of second ********** " is an approach of adding lipase in raw sake in the white-distilled-liquor manufacture by the non-cooking raw material, and improving generation of an unpleasant flavor peculiar to non-cooking fermentation.
[0013] Moreover, the example which uses lipase in raw material processing is also reported. That is, although "the distilling methods (JP,58-35674,B), such as sake which adds lipase at the time of immersion of rice,", "the manufacturing method (JP,62-55069,A) of the alcoholic beverage which uses cone GURITTSU as a raw material", and "the manufacture approach (JP,8-214863,A) of the alcoholic beverage by the liquefaction brewing method" are mentioned, the lipase by which each of these was added in the phase of the main fermentation in raw sake has deactivated.
[0014] Therefore, the effectiveness of this invention that each approach of applying the lipase known from the former to a brewing raises the rate of the dissolution of the steamed rice in low-temperature fermentation is not expectable.
[0015] Furthermore, there is completely nothing, this invention persons perform a test brewing for the first time using nuclease, and report of using the nuclease which is the active principle of the enzyme agent for a brewing of this invention in a brewing clarifies the remarkable effectiveness.
[0016] This invention is further explained to a detail. Although the lipase which can be used for this invention is an enzyme which decomposes a lipid, for example, the triglyceride lipase which carries out the catalyst of the reaction which hydrolyzes a triglyceride to a glycerol and a fatty acid gradually is shown, all can be used if it is the lipase agent known conventionally.
[0017] For example, Lipase M (trade name: Mucor sp. origin), Lipase F, Lipase D (all trade name : Rhizopus sp. origin), Lipase A Lipase 49 (all trade name : Aspergillus oryzae origin), Lipase AY, Lipase L10 (all trade name : Candida sp. origin), Lipase PC, Lipase AK (all are the trade name-seudomonus sp. origin), Lipase G, LipaseRF (all are the trade name-enicillium sp. origin), etc. are mentioned. The lipase M of the Mucor group origin and the lipase F of the Rhizopus group origin are used more preferably.
[0018] The nuclease which can be used for this invention is an enzyme which carries out the catalyst of the reaction which acts on the nucleotide and nucleoside which are a nucleic acid and its decomposition product in hydrolysis, and all can be used if it is the nuclease agent known conventionally.
[0019] For example, nuclease "Amano" (trade name-enicillium citriunum origin) is mentioned.
[0020] Furthermore, when using it as substitution of koji, alpha-amylase, glucoamylase, and acid protease are used together by lipase and/or nuclease, and acid phosphatase etc. is more preferably used together.
[0021] Although the alpha-amylase of what kind of the origin is sufficient as alpha-amylase, the biodiastase (trade name: Aspergillus oryzae origin) marketed can obtain easily.

[0022] Moreover, although the glucoamylase of what kind of the origin is sufficient as glucoamylase, glucoamylase "Amano" (trade name: Aspergillus niger origin) is desirable.
[0023] Moreover, although the acid protease of what kind of the origin is sufficient as acid protease, newlase (trade name: Rhizopus origin) is used effectively.
[0024] Moreover, although the acid phosphatase of what kind of the origin is sufficient as acid phosphatase, the acid phosphatase (P-3627, wheat wheat bran origin) of the sigma company of a commercial item can obtain easily. Moreover, the acid phosphatase which cultivated the yeast of mold, such as an Aspergillus group, and a Saccharomyces group etc. by the nutrition culture medium, produced acid phosphatase, and was refined with various chromatographies is sufficient.
[0025] As amount of the various above-mentioned enzyme agents used, the lipase used for this invention can use one to 500 unit, and nuclease can use more preferably 0.1 or more units per 1g of white rice of 0.1 or more units per 1g of white rice of one to 200 units.
[0026] Moreover, as for alpha-amylase, it is [the enzyme agent used together] desirable [as for 500 or more units and acid phosphatase] per 1g of white rice for 100 or more units and glucoamylase to use 40 or more units, and for acid protease to use ten to 40 unit.
[0027] However, since the difference in a raw material and the difference of fermentation conditions (temperature etc.) change with an area or works, these amount used has texture ****** desirable in the use unit of each enzyme according to each based on an experiment.
[0028] Especially the analysis method used by this invention is as having been shown below, unless it indicates. In measurement of enzyme activity, alpha-amylase, glucoamylase, acid protease, and acid carboxypeptidase activity follow a National Tax Administration Agency predetermined analysis method. Acid phosphatase activity is the TORRIANI method (Biochem.Biophys.Acta, 38 volumes, 460 pages (1960)). Lipase activity is the approach of making one unit the amount of enzymes which emulsification olive oil is made [amount] into a substrate and separates the fatty acid of 1micromole in 1 minute. Nuclease activity measured the amount of enzymes which ribonucleic acid sodium is made [amount] into a substrate and carries out 0.001 ****s of the absorbances in 260nm in 1 minute by the approach of making it into one unit.
[0029] The component analysis of production alcohol is a phenol sulfuric-acid method about all sugar, and is glucose B Test Wako (Wako Pure Chem make) about a glucose, and whenever [acidity and amino acid] measured whenever [alcohol content and sake] according to the National Tax Administration Agency predetermined analysis method.
[0030] the rate of the dissolution, and saccharification -- the rate and the rate of fermentation were computed according to Iwano's and others approach (the Brewing Society of Japan magazine, 82-volume 661 pages (1987)).
[0031] About i-amyl alcohol (it is hereafter described also as i-AmOH), isoamyl acetate (it is hereafter described also as i-AmOAc), and ethyl hexoate (it is hereafter described also as EtOCap), it measured by the gas-chromatography method.
[0032] The enzyme agent used in the following examples is as follows. In alpha-amylase, biodiastase (trade name: Aspergillus oryzae origin) and glucoamylase used glucoamylase "Amano" (trade name: Aspergillus niger origin), and acid protease used newlase (trade name: Rhizopus group origin). Moreover, acid phosphatase used the thing of the Aspergillus oryzae origin. As lipase, Lipase M (trade name: Mucor group origin : more than 10,000 units / g) and Lipase F (trade name: Rhizopus group origin : more than 150,000 units / g) were used, and nuclease "Amano" (trade-name-enicillium group origin: more than 13,000 units / g) was used as nuclease (all are the Amano Pharmaceuticals company make).
[0033] An example is given to below and this invention is concretely explained to it. This invention is not limited to the following examples and can make the usual change in the technical field of this invention.
[0034]
[Example]
Example 1 Lipase, the lipase to the steamed rice dissolution in the addition effectiveness low-

temperature fermentation of nuclease, and the addition effectiveness of nuclease were investigated. Raw material rice used the white rice of Yamada Nishiki (from Hiroshima Prefecture), and 50% of rice-cleaning rates, and performed it by the training in three step of the 100g of the total rice by the brewing combination shown in Table 1.

[0035]

[Table 1]

	添	仲	留	合計
総米	20	30	50	100 g
掛米	15	25	40	80 g
麹	5	5	10	20 g
くみ水	25	40	65	130 ml

[0036] In addition, enzyme agent preparation used all the malted rices of Table 1 as kake-mai. Adding 1ml of brewing processing water which contains a lactic acid in the phase of ********, yeast added 0.5ml per 1 preparation of association No. 9 ampul yeast (Brewing Society of Japan make).

[0037] Enzyme agent preparation was blended so that it might become alpha-amylase 100 unit, glucoamylase 50 unit, and acid protease 500 unit per 1g of white rice as a basic enzyme agent, and it added 0.1% of lipase and nuclease of ten units and the total rice for acid phosphatase to the basic enzyme agent. Fermentation temperature performed low-temperature fermentation fixed 10 degrees C. Solid liquid separation was performed by centrifugal separation after fermentation termination, and the component of production alcohol was analyzed. The result is shown in Table 2.

[0038]

[Table 2]

仕込区分						推定粕歩合(%)	純アルコール収得量(L)
麹	基本酵素剤	酸性ホスファターゼ	ヌクレアーゼ	リパーゼM	リパーゼF		
○						54.8	337
	○					83.8	247
	○			○		48.0	352
	○				○	47.6	328
	○		○			54.4	332
	○	○				76.4	279
	○	○		○		42.6	365
	○	○			○	45.0	351
	○	○	○			45.3	354
	○	○	○	○		40.5	360
	○	○	○		○	42.3	363

[0039] When basic enzyme agent preparations are 83.8% of ******, and amount of absolute alcohol acquisition 247 L/t and Lipase M, Lipase F, and nuclease were added to the basic enzyme agent compared with 54.8% of ****** of koji preparation, and amount of absolute alcohol acquisition 337 L/t, respectively, it became koji preparation, an EQC, or a value beyond it, and big effectiveness was accepted, so that more clearly than Table 2. Especially the effectiveness of lipase addition was large and became a value beyond koji preparation.

[0040] On the other hand, only effectiveness with the slight preparation which added acid phosphatase to the basic enzyme agent was accepted. When seen about the synergistic effect of three persons of acid phosphatase, lipase, and nuclease, the synergistic effect of acid phosphatase, lipase and acid phosphatase, nuclease and nuclease, and lipase was accepted.
[0041] Example 2 Lipase and nuclease were added to this by using as the base the enzyme agent which blended acid phosphatase 10 unit at the basic enzyme agent given in lipase and the related example 1 of the addition of nuclease, and the steamed rice dissolution, like the example 1 publication, the small brewing trial was performed and the relation of the addition and steamed rice dissolution was investigated.
[0042] As the result was shown in drawing 1 and drawing 2 , effectiveness was notably accepted by 0.025% or more of addition to the total rice.
[0043] Example 3 The effect lipase and nuclease addition which are exerted on lipase and the production alcohol component of nuclease addition investigated by performing the small brewing trial of the 1kg of the total rice about the effect affect a production alcohol component. Brewing combination, the brewing processing water addition, and the yeast addition were made into the amount of 10 times of an example 1, the total rice added lipase and nuclease 0.05% to this, respectively by having considered the enzyme agent which blended acid phosphatase with the basic enzyme agent as contrast, and fermentation temperature performed low-temperature fermentation fixed 10 degrees C.
[0044] It fermented for 30 days, and solid liquid separation was performed by the ****** method, and component analysis and organic-functions evaluation were performed about the obtained production alcohol. Organic-functions evaluation was performed by the grading method (five points law) by 13 panelists. A result is shown in Table 3.
[0045]
[Table 3]

	麹仕込み	基本酵素剤 酸性ホスファターゼ 配合	リパーゼ添加	ヌクレアーゼ添加
アルコール(%)	17.6	14.7	18.7	17.7
日本酒度	-10	-30	-22	-25
酸度	2.4	2.7	2.5	3.0
アミノ酸度	1.8	1.7	1.6	2.3
全糖(%)	5.5	8.7	7.2	8.4
グルコース(%)	1.7	7.1	6.0	7.1
純アルコール収得量(L/t)	312	261	343	325
粕歩合(%)	59	65	27	38
i-AmOH(ppm)	160	154	121	98
i-AmOAc(ppm)	12.4	6.6	7.2	4.6
EtOCap(ppm)	2.5	2.4	1.9	1.5
E/A	7.8	4.3	6.0	4.7
官能評価	3.0	3.0	2.5	2.8

[0046] Lipase and nuclease addition preparation have the good dissolution of a steamed rice so that more clearly than Table 3. That is, ****** is decreasing clearly with 27 - 38% to ****** in ****** being 59%. Moreover, whenever [acidity or amino acid] was comparable, the glucose content was high, isoamyl alcohol was low, and ester was comparable. Organic-functions evaluation had the best lipase addition, and, subsequently to nuclease addition, effectiveness was seen a little.
[0047] Example 4 The steamed rice dissolution effectiveness in low-temperature fermentation was examined using the lipase agent of the steamed rice dissolution effectiveness various microorganism origins by various lipase agent addition.
[0048] The steamed rice dissolution trial system using 5g of hot-air-drying precooked rice is shown in Table 4.
[0049]
[Table 4]

熱風乾燥α米	5.0 g
基本酵素溶液	1.0 ml
リパーゼ溶液	1.0 ml
乳酸緩衝液 (0.1M,pH4)	1.0 ml
酵母液	0.1 ml
蒸留水	7.0 ml

[0050] In addition, hot-air-drying precooked rice was prepared in Yamada Nishiki of 50% of rice-cleaning rates. The basic enzyme agent was blended so that it might become alpha-amylase 100 unit, glucoamylase 50 unit, acid protease 500 unit, and acid phosphatase 15 unit per 1g of white rice. Lipase used each lipase 5mg per one experimental plot using 11 kinds of commercial lipase agents. 0.1ml (Brewing Society of Japan make) of association No. 901 ampul yeast was used for yeast, and it performed fixed period fermentation at 10 degrees C using the conical tube (falcon company make) of 50ml **. An analysis result is shown in Table 5.

[0051]

[Table 5]

商品名	起源	溶解率 (%)	糖化率 (%)	発酵率 (%)	純アルコール収得(l/t)	粕歩合 (%)
基本酵素剤		57.3	86.8	55.6	182	74
Lipase M	Mucor sp.	74.9	80.3	95.9	380	30
Lipase F	Rhizopus sp.	73.2	89.4	82.0	353	35
Lipase A	Asp. oryzae	67.2	86.4	77.5	296	50
Lipase AY	Candida sp.	67.0	80.2	87.8	310	50
Lipase L10	Candida sp.	64.6	85.0	58.1	210	56
Lipase PC	Pseudomonus sp.	63.3	85.1	56.7	201	59
Lipase D	Rizopus sp.	63.6	97.0	55.5	225	59
Lipase AK	Pseudomonus sp.	63.7	85.9	63.1	227	58
Lipase 49	Aspergillus sp.	63.6	84.5	72.8	257	59
Lipase G	Penicillium sp.	62.4	80.4	81.2	268	62
Lipase RF	Penicillium sp.	62.0	85.2	66.8	232	63

[0052] Compared with the basic enzyme agent division which does not add lipase, by the lipase additive area, ****** showed the low value and it became clear that any commercial lipase agent has effectiveness in the dissolution of the steamed rice in low-temperature fermentation. Especially, remarkable effectiveness was accepted in the lipase M of the Mucor group origin, and the lipase F of the Rhizopus group origin.

[0053]

[Effect of the Invention] In brewing which used the enzyme agent for a brewing of this invention as reinforcement or substitution of koji Improvement in brewing and the EQC using usual careful distillation koji or the raw material utilization factor beyond it, and quality is realizable. By use of this enzyme agent The effectiveness which manufacture of high-class sake, such as the effectiveness and quality sake brewed from the finest rice which make the labor of careful distillation koji structure mitigate, and junmai-shu, becomes easy, and can increase production, and the effectiveness of reducing a manufacturing cost further are attained.

[Translation done.]